THE USE OF ALGAL ASSAYS TO EVALUATE ALGAL BIOMASS IN REGULATED STREAMS

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by

Jennifer Mae Carr

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by

Jennifer Mae Carr

Approved by Committee:

Dr. Wayne B. Merkley, Chairperson

Dr. Phyllis J. Kingsbury

Dr. Lawrence A. Funke

Dean of the School of Graduate Studies

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An abstract of a Thesis by
Jennifer Mae Carr
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Advisor: Dr. Wayne B. Merkley

The problem. This study was undertaken to evaluate the algal growth potential of the Des Moines River as it passes through Saylorville and Red Rock Reservoirs.

Procedure. The test organism, Selenastrum capricornutum Printz, was grown in an algal assay which used various additions of nutrient spikes to waters collected above and below each reservoir. Seasonal river samples were collected from December 1978 to October 1979. Growth response was measured gravimetrically.

Findings. Addition of trace metals in combination with iron and manganese most frequently increased algal growth at all sampling stations, followed by the addition of trace metals in combination with phosphorus and nitrogen. Actual algal yield correlated with predicted algal yields using phosphorus and nitrogen content of the waters at all stations during most sampling periods. Using N:P ratios, phosphorus is most often the limiting factor in the river. Above- and below-reservoir differences in algal productivity were not distinct. Samples taken from the station below Saylorville Reservoir responded more frequently to nutrient additions than did samples taken from the corresponding above-reservoir station. Differences above and below Red Rock Reservoir (below the City of Des Moines) were less distinct.

Conclusions. Nutrient spike additions to Des Moines River water did not consistently increase algal yield, although actual yield correlated with predicted algal yield. Above- and below-reservoir differences were not distinct. Nutrient additions more frequently increased algal yield at the stations above the City of Des Moines than below it.

Recommendations. Further study should include a year-round survey using more sampling sites and frequent collections to determine the algal growth potential and serve as a basis for predicting future trends. Use of individual nutrient spikes, rather than combinations of nutrients, would be useful. Adding a few sampling sites along the Raccoon River would be helpful to determine where algal growth potential changes occur in the downstream river. Use of EDTA to chelate heavy metals prior to assay could be recommended.

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INTRODUCTION

Phytoplankton are primary producers in the aquatic community and, as such, create the base of aquatic food chains. Because of their importance, their growth response to a substance, or group of substances, needs to be ascertained. Since the process of eutrophication is the most important pollution problem affecting U.S. lakes and rivers (Hutchinson, 1973), an accepted, uniform testing procedure is necessary to determine eutrophication standards.

During the past twenty years, bioassays have become basic tools for the detection, evaluation, and abatement of eutrophication and water pollution. They have been used in studies that determine (1) the suitability of environmental conditions for aquatic life, (2) the favorable and unfavorable concentrations of levels of environmental factors, such as dissolved oxygen, for aquatic life, (3) the effectiveness of different waste treatment methods, (4) water quality requirements for aquatic life, and (5) compliance with water quality standards, effluent requirements, and discharge permits, to name a few examples (American Public Health Association, 1975).

Algal assays are those varieties of bioassays which utilize varying algal species as test organisms. Algal assays typically consist of three steps: (1) selection and measurement of appropriate factors or conditions during the assay (such as total cell carbon which indicates algal biomass),

(2) presentation and statistical evaluation of measurements made during the assay, and (3) interpretation of the results with respect to the specific problems being investigated (American Public Health Association, 1975). The specific experimental design of each algal assay must be tailored to meet the actual situation. It is necessary that all the pertinent environmental factors be considered in the planning of an algal assay to insure that valid results and conclusions are obtained. It may, for example, be of value to sample both the epilimnion and hypolimnion in stratified bodies of water because these areas can significantly differ in certain water quality parameters, such as dissolved oxygen, orthophosphate and nitrogen content (Wetzel, 1975).

Recently, there has been marked interest in quantitative laboratory techniques for the assessment of the trophic status of lakes, for the evaluation of limiting or surplus concentrations of critical elements, and for the prediction of the behavior of a given body of water in response to increased or decreased nutrient load (Chiudani and Vighi, 1974). With the surge of interest in the growing problem of eutrophication, the Joint Industry/Government Task Force on Eutrophication recognized that acceptable standardized algal growth tests needed to be developed as a tool in controlling eutrophication. While many scientists had improvised algal assays to meet their specific needs, these assays did not offer reproducible results between different laboratories

or different geographical areas (Environmental Protection Agency, 1971).

In February 1969 the Joint Task Force published the Provisional Algal Assay Procedure (PAAP). The PAAP was developed from the collective knowledge and experience of persons who had fundamental knowledge of algal physiology, algal growth responses, and experience with algal assays of various types. Shortly after publication of the PAAP, a group consisting of government, university and industrial laboratories undertook a comprehensive research program to improve and evaluate it. In their investigation (Weiss and Holmes, 1971), the growth response of the test alga was compared in various dilutions of the recommended synthetic culture medium, using Selenastrum capricornutum Printz as the test organism. Regression analyses comparing nutrient strength with algal yield indicated that growth responses closely related to nutrient strength were produced at each participating laboratory. It was also determined that descriptive algal assays (such as those estimating the total number of cells that may be grown under standard conditions) have poor accuracy. It was found that comparative algal assays, such as those used to estimate the differences between biomasses which would be produced under two separate sets of conditions, were quite precise. Small differences in nutrient strengths of media could be detected. At the time the study was conducted, comparisons between

laboratories were of a lower order of precision. The Algal Assay Procedure: Bottle Test (E.P.A., 1971) was developed as a result of the intensive investigation of the PAAP. A more exacting standard algal assay procedure has very recently been suggested (Miller et al., 1978), which will presumably diminish differences between separate laboratory algal assays.

The Algal Assay Procedure: Bottle Test (E.P.A., 1971) and the Selenastrum capricornutum Printz Algal Assay Procedure: Bottle Test (Miller et al., 1978) are based on Liebig's Law of the Minimum which states that growth is limited by the substance that is present in the smallest quantity with respect to the physiological needs of the organism. The test in its present form is intended for use in the following general situations: (1) assessment of a receiving water to determine its nutrient status and sensitivity to change; (2) evaluation of materials and products to determine their potential effects on algal growth in receiving waters; and (3) assessment of effects of changes in waste treatment processes on receiving waters. Specifically, it is intended that the test be used: (1) to identify growth-limiting nutrients; (2) to determine the biological availability of algal growth-limiting nutrients; and (3) to quantify the biological response to changes in concentrations of algal growth-limiting nutrients. These measurements are made by adding Selenastrum capricornutum Printz to the test

water and determining algal growth at appropriate intervals. The test may also be used to determine whether or not various compounds or water samples are toxic or inhibitory to algae.

Many studies have been conducted to examine further uses of the algal assay procedure, as well as to determine the eutrophic status of certain lakes and rivers.

In the fall of 1970 a program was initiated to conduct a series of ongoing algal assays from several lakes on a quarterly basis (Maloney et al., 1971) in order to determine the effects of seasonal changes on the ability of the waters to support algal growth. This study also attempted to correlate the chemistry of the waters with their ability to support algal growth, and to evaluate the potential effects on algal growth of the addition of various nutrients to the waters.

Algal assays were conducted on the Snake River and its tributaries (Greene et al., 1975a) with three purposes in mind: (1) to determine if algal growth was consistent with results predicted from review of chemical analyses for orthophosphate and total soluble inorganic nitrogen; (2) to determine if algal yields were limited by phosphorus, nitrogen, or some other nutrient essential to algal growth; and (3) to predict the effects of phosphorus and nitrogen additions on algal productivity. They found a high degree of correlation between the expected trophic state of the 18

Snake River basin sampling sites and their algal productivity response. This high correlation indicated that the Algal Assay Procedure: Bottle Test (E.P.A., 1971) is sensitive to the subtle changes of nutrient content in the various river waters assayed. It was also found that phosphorus was an algal growth-limiting factor in 45% of the sites tested and that river waters with high concentrations of orthophosphate are more likely to become algal growth-limited by nitrogen or some other essential nutrient.

Algal assays were also performed on the Spokane River system (Miller et al., 1975). Nutrients enter the Spokane River basin from several major sources including domestic, industrial, agricultural and groundwater intrusion. Population increases and lack of adequate wastewater treatment facilities had been identified as the major causes of the increased amounts of nitrogen and phosphorus that entered the system. The assessment of eutrophication in the Spokane River system was complicated by the occurrence of heavy metals, such as zinc.

Conclusions derived from the use of algal assays on the Spokane River system were: (1) zinc regulates algal growth in the river from Post Falls, Idaho, to Riverside State Park, Washington (a distance of approximately 50 miles), and addition of phosphorus in this area would do little to increase algal growth unless the zinc concentrations were reduced; (2) a natural reduction of zinc at the Spokane

sewage treatment plant at Long Lake Dam enabled the algal growth to increase proportionately to the orthophosphate content of the water; and (3) assessment of nutrient enrichment problems, complicated by the occurrence of heavy metals, can be accomplished by the use of algal assays.

Chiudani and Vighi (1975) used algal assays with Selenastrum capricornutum Printz to evaluate the trophic status of lakes by ascertaining the effective availability of the nutrient elements in order to seek out the principal factors responsible for algal growth and to study the dynamics of possible nutrient limitations during an annual cycle.

Gerhold and Otto (1976) conducted a study at a proposed nuclear generating site near Burlington in Coffee County, Kansas, where cooling water for the power plant would be supplied by a 5,000-acre lake to be constructed at the site. In using algal assays in this study, their objectives were to determine: (1) limiting nutrients in the John Redmond Reservoir, an impoundment on the Neosho River, which would supply water for the cooling pond; (2) limiting nutrients in the Neosho River; (3) biological availability of the limiting nutrients in the river and reservoir versus concentrations obtained by chemical analyses; (4) sensitivity to change in the amount of algal growth supported by the various waters in response to supplemental, specific nutrient inputs; and (5) the effects of evaporative concentration of nutrients on algal growth within the proposed cooling

pond. Results obtained from this study indicated: (1) nitrogen or phosphorus alone or in combination were the limiting nutrients, and varied depending on the time of year the sample was taken; and (2) chemical analysis of the inorganic nutrient forms frequently underestimated the biological availability of these nutrients. It was concluded that chemical analyses alone should not be substituted as estimators of biological nutrient availability.

Gargas (1978) studied the effects of sewage on algal growth using algal assays to determine the influence of sewage loading on bodies of water as well as to predict the effects of sewage treatment. It was found that spiking with sewage to natural aquatic ecosystems caused an increase in the production of algae.

Heavy metals are often present in river waters and have been found to inhibit algal productivity. Bartlett et al. (1974) studied the effects of copper, zinc and cadmium on Selenastrum capricornutum. Results indicated that combinations of copper, zinc and cadmium were similar in toxicity to equal concentrations of zinc. Combinations of copper and cadmium resulted in a greater growth rate than equal concentrations of copper, suggesting that cadmium inhibits copper toxicity.

Chiudani and Vighi (1978) used algal assays to verify the sensitivity of S. capricornutum to heavy metal toxicity. It was found in their study that this alga shows extreme

sensitivity to heavy metals and could thereby be considered an excellent test organism for the evaluation of extent of toxicity.

Christensen and Scherfig (1979) conducted algal assays to observe the responses of S. capricornutum to manganese, copper and lead added singly or in combination to both artificial media and natural waters. A 50% reduction in the total algal cell volume of S. capricornutum in standard algal medium was found to have occurred with the addition of either 3.1 µg manganese/l, 85 µg copper/l, or 140 µg lead/l.

The presence of heavy metals in the Des Moines River has been noted as being one of its water pollution problems by the Iowa Department of Environmental Quality in its 1975 Iowa Water Quality Report. Other problems occurring in this basin are: (1) high turbidity and suspended solids; (2) violation of dissolved oxygen and ammonia standards; and (3) concentrations of pesticides in violation of Iowa Water Quality Standards.

Baumann et al. (1980) examined levels of arsenic, barium, cadmium, chromium, lead and silver in the Des Moines River basin. Barium was detected at every station examined along the river, although the primary drinking water standard of 1.0 mg/l was never exceeded. Cadmium was detected during this study on two occasions but on both occasions concentrations were very low. Chromium was also found to be present

at every station in low quantities. The concentration of total lead approached the Iowa Department of Environmental Quality state standard of 0.10 mg/l on several occasions, while the primary drinking water standard of 0.05 mg/l for soluble lead was equalled or exceeded at several stations. Concentrations of silver and arsenic were found to be very low throughout the study period and never approached state drinking water or stream standards.

The nutrient and suspended algae levels in central Iowa streams were examined by Kilkus et al. (1975). Nutrient levels, with surface runoff as the major contributor, were so high that nitrogen and phosphorus were not limiting to phytoplankton growth which seemed to be held in check by physical factors (i.e., turbidity, light penetration, temperature, flow).

Arneson (1977) used algal assays to compare various combinations of deicing salt and sewage effluent both diluted with Des Moines River water. The conclusion reached was that the addition of both sewage effluent and deicing salt to Des Moines River water had a growth enhancing effect on the test alga, Selenastrum capricornutum.

Assessment of water pollution problems in the Des Moines River basin is complicated by the presence of two main-stem dams, Saylorville and Red Rock. One of the consequences of impounding large prairie rivers is the settling out in reservoirs of materials that would normally

remain suspended in the rapidly moving water. Thus, the water leaving a reservoir of this kind would be expected to have some alteration of its physical and chemical characteristics.

Baumann et al. (1980) have shown some striking differences in water quality parameters in the Des Moines River above and below Saylorville and Red Rock Reservoirs. Turbidity was greater at the stations above each dam than it was downstream. Nitrogen and phosphorus were present in greater quantities at the above-dam stations than at the below-dam stations. Specific conductance, carbon dioxide, total alkalinity and total hardness were greater at the above-dam locations than they were at the stations downstream of each dam. There was less dissolved oxygen found in the waters upstream of each dam than there was downstream.

Little research has been conducted up to this point to determine the effects of reservoirs on downstream water quality with respect to algal productivity. This project is concerned with measuring the algal growth potential of the Des Moines River before and after passing through Saylorville and Red Rock Dams in an attempt to show the effects on the growth of planktonic algae of the water quality changes that occur in the water as it moves downstream.

The objectives of this project are to determine:

(1) the nutritional status of the Des Moines River

system;

(2) the critical nutrient(s) responsible for the support of algal growth within the river system; and

(3) the effects Saylorville and Red Rock Dams have on the potential algal biomass in the downstream river.

MATERIALS AND METHODS

Four sampling sites (Figure 1) were used to represent the varying types of water quality with respect to the impact of Saylorville and Red Rock Reservoirs on downstream water quality: Station 1 was upstream from Saylorville Reservoir near the Highway 30 bridge west of Boone; Station 2 was downstream from Saylorville Dam, adjacent to Sycamore Park, north of Des Moines; Station 3 was upstream from Red Rock Reservoir near the Highway 46 bridge southeast of Des Moines; and Station 4 was downstream of Red Rock Dam near the County Road T-15 bridge, south of Pella.

Surface samples were taken at each of the four stations, beginning at Station 1 and continuing through Station 4 to insure continuity in sampling. Samples were collected seasonally (Table 1) using a plexiglass Kemmerer. Each sample was then transferred to a 3.8 liter wide-mouth polypropylene container and transported at 4°C in dark conditions.

To allow use of unialgal test species, indigenous

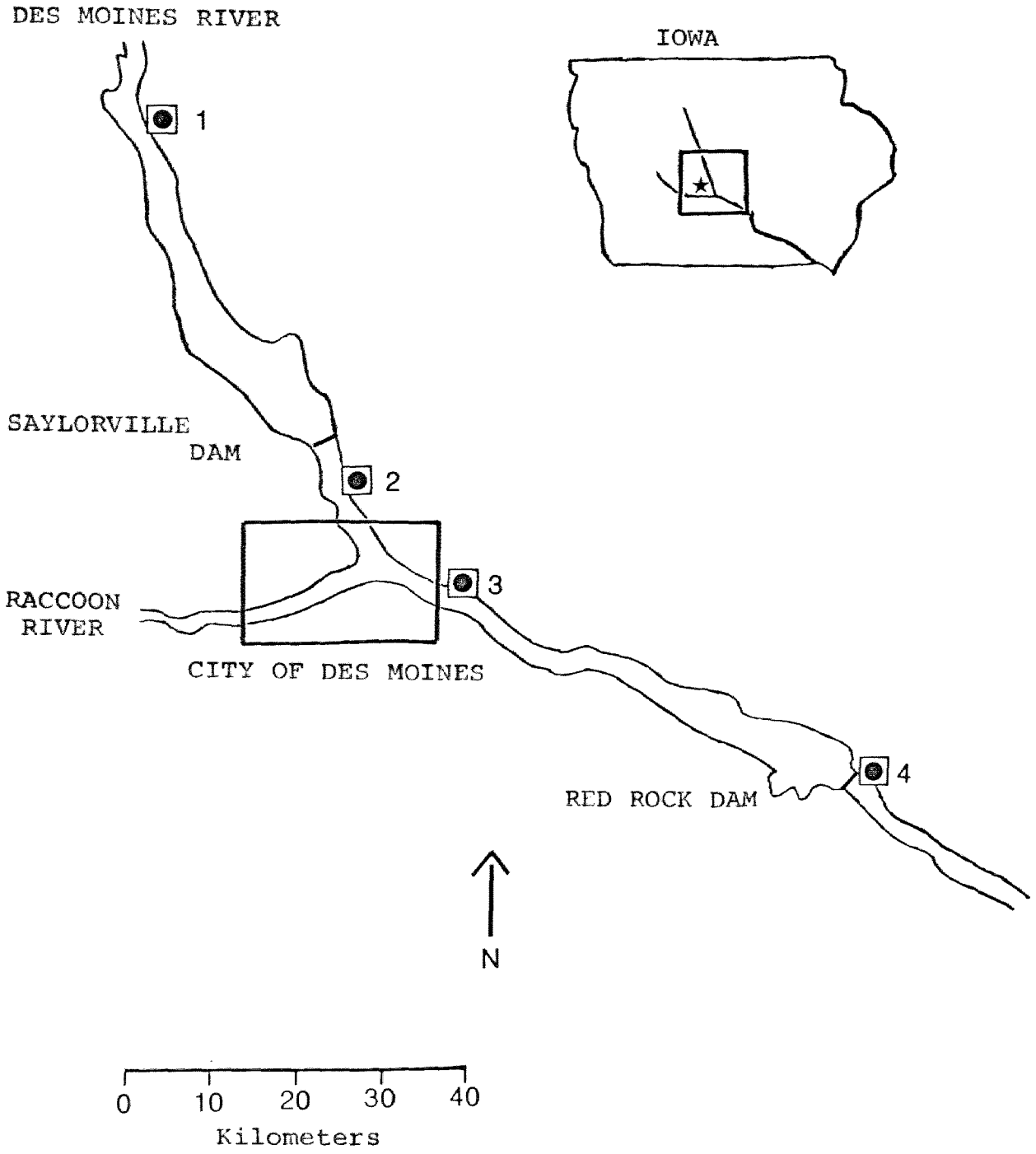

Figure 1. Location of sampling stations.

Table 1. Dates of sample collections, representative seasons and corresponding water temperatures.

Station	Date Collected	Season	Water Temp. (°C)*
1	Dec. 1, 1978	Winter	0.0
2	" " "		2.0
3	Dec. 14, 1978		2.0
4	" " "		3.0
1	May 25, 1979	Spring	15.5
2	" " "		15.5
3	May 26, 1979		16.5
4	" " "		17.0
1	Sept. 8, 1979	Summer	20.5
2	" " "		--
3	" " "		24.0
4	" " "		24.0
1	Oct. 19, 1979	Fall	10.8
2	" " "		12.8
3	" " "		20.5
4	" " "		12.0

* - as determined by Baumann et al. (1980)

organisms (including algae) were lysed by autoclaving the river water samples at 121°C and 1.1 kg cm^2 for 30 minutes, cooled, and stored in the dark at 4°C until used.

Glassware preparation followed the recommended procedure (Miller et al., 1978). All cylinders, flasks, bottles, centrifuge tubes and vials were washed with a non-phosphate detergent and rinsed thoroughly with tap water. This was followed by a rinse with a 10% solution (by volume) of reagent grade hydrochloric acid (HCl); vials and centrifuge tubes were filled with the 10% HCl solution and allowed to remain a few minutes; all larger containers were filled to about one-tenth capacity with HCl solution and swirled so that the entire inner surface was bathed. After the HCl rinse, the glassware was neutralized with a saturated solution of Na_2CO_3, then rinsed five times with tap water followed by five rinses with glass-distilled water.

Cleaned glassware was dried at 50°C in an oven and stored in closed cabinets with the tops covered with aluminum foil. Autoclavable glassware, such as centrifuge tubes and culture flasks, was autoclaved for 15 minutes at 121°C and 1.1 kg cm^2, cooled, and stored in closed cabinets.

The recommended test alga for the Selenastrum capricornutum Printz Algal Assay Procedure: Bottle Test (Miller et al., 1978), Selenastrum capricornutum Printz, was obtained from the Environmental Protection Agency, Corvallis Environmental Research Laboratory, Special Studies

Branch, 200 S.W. 35th St., Corvallis, Oregon 97330. Taxonomically, S. capricornutum belongs in the Chlorophyceae (green algae), Order Chlorococcales, Family Selenastraceae. It is characteristically tolerant of organic pollution and is the indicator organism of choice in many current algal assays.

A stock culture was prepared by asceptically transferring a 1 ml portion of the inoculum species to 50 ml of culture medium in a 250-ml Erlynmeyer flask. A biweekly stock transfer was performed to insure an adequate supply of "healthy" cells. Two-week old cultures were used as the source of the test inoculum.

Cells from the stock culture were centrifuged (using 15 ml of culture in 15 ml centrifuge tubes) for 5 minutes at 1000 rpm, and the supernatant discarded. The sedimented cells were then resuspended in 15 ml of glass-distilled water containing 15 mg $NaHCO_3$/l, and again centrifuged for 5 minutes at 1000 rpm. The supernatant was discarded and the cells were resuspended in 2 ml of the water-bicarbonate solution and used as the inoculum.

Initial cell counts were determined using a hemacytometer. The appropriate volume of inoculum was then added to each culture flask to give a final cell concentration of approximately 1000 cells per milliliter.

The recommended incubation conditions for S. capricornutum are 24 ± 2°C under continuous cool-white

fluorescent lighting at 4304 lumens (400 ± 10% foot-candles) (E.P.A., 1971). The incubation conditions used in this study were modified somewhat. The incubator used was determined to have 450 foot-candles of illumination using a Weston Illumination Meter, model 756, and the temperature inside the incubator (Precision Thelco Incubator, Model 818) was 23°C. Due to limitations of the timing device on the incubator, the illumination period was not continuous but was for 18 hours, followed by 6 hours of darkness.

To insure the availability of carbon dioxide in the culture flasks, the pH was maintained below 8.5 by: (1) using optimum sample to volume ratios, recommended to be 50 ml of sample water in a 250-ml Erlynmeyer flask (E.P.A., 1971); (2) shaking each culture flask once daily; and (3) using foam plugs to permit good gas exchange and prevent contamination.

Flasks were numbered permanently so that anomalous growth related to a specific flask could be identified.

The parameter chosen to describe growth of the test alga was maximum standing crop expressed as dry weight. Maximum standing crop (defined as the maximum algal biomass achieved during incubation) has been achieved when the increase in biomass is less than 5% per day (E.P.A., 1971). The maximum standing crop obtained in the incubator used was determined to occur on the seventh day of incubation (Figure 2). After this maximum standing crop was achieved,

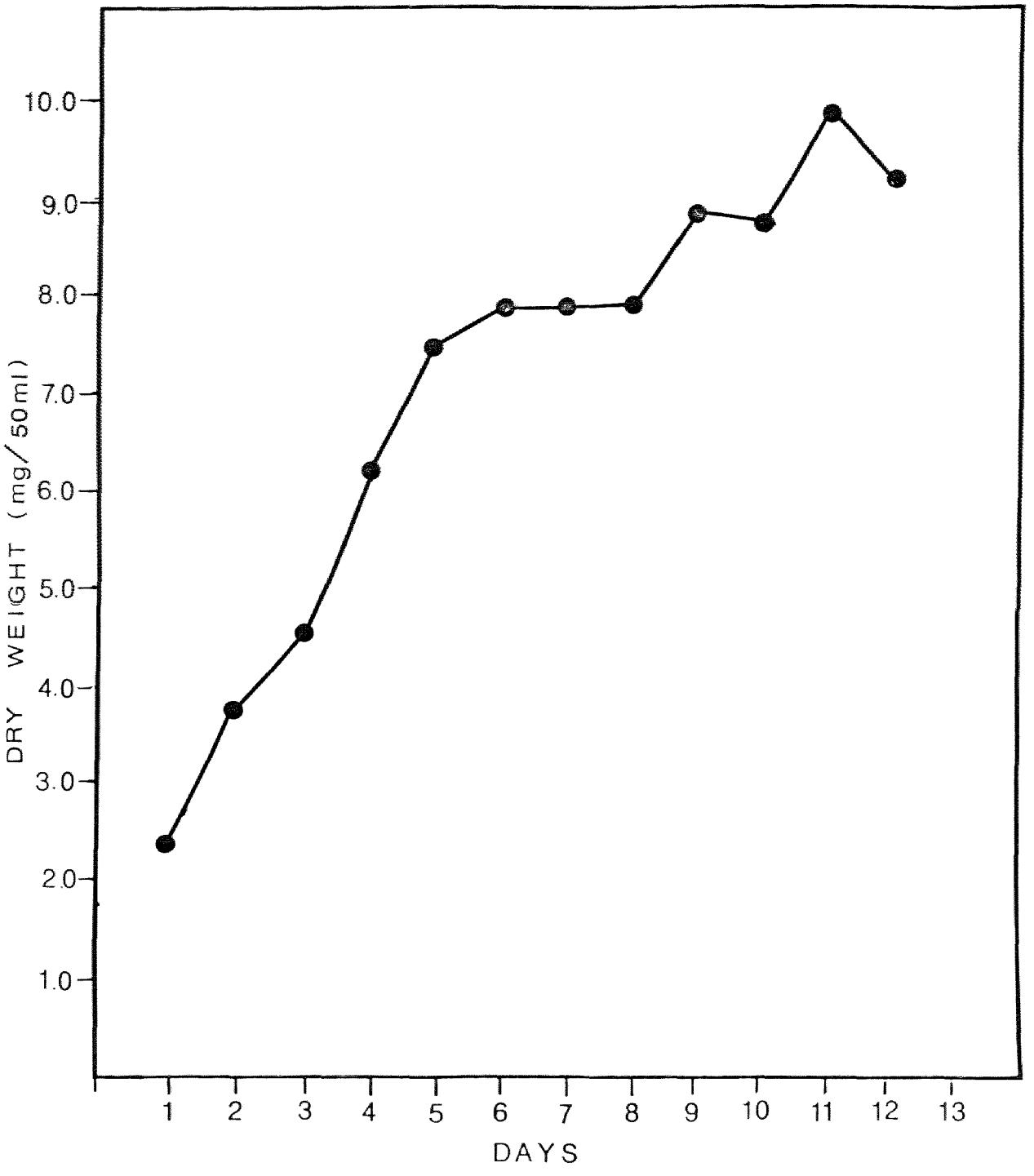

Figure 2. Average dry weight (mg/50 ml) of three replicate flasks obtained during a pilot study to determine day of maximum algal growth in incubation chamber.

the algal biomass was measured gravimetrically, using the recommended Method II (Miller et al., 1978).

Fifteen milliliters of the algal culture was filtered through a tared Millipore filter, type BD with a 0.60 micrometer pore size. Filters were protected and kept separate from each other by placing them in small aluminum-foil dishes. Before weighing initially, filters were dried for 2 hours at 70°C in an oven, and then cooled in a desiccator for 1 hour. The 15 ml aliquot of algal culture was filtered under a vacuum pressure less than 1.1 kg cm^2. The filter and filter funnel were rinsed with 50 ml of glass-distilled water using a wash bottle. The filters containing algae were then dried to constant weight for 4 hours at 70°C in an oven, cooled in a desiccator for 1 hour, and weighed.

To assess the impact of the reservoirs upon the growth of algae, the possible growth-limiting nutrients were divided into four groups: (1) trace metals (boron, zinc, cobalt, copper, molybdenum); (2) iron and manganese; (3) phosphorus and nitrogen; and (4) macronutrients (calcium, magnesium, chloride, sulfur, sodium). These four groups were then added alone and in all possible combinations to samples from each of the four stations, and the maximum standing crop was determined. Two replicates were made for each of the combinations tested (i.e., a total of 32 tests for each water sample tested).

The results were analyzed as a 2 x 2 x 2 x 2

factorial experiment. The factorial layout used is shown in Table 2. The computer program used for analyzing the results can be found in Appendix 11.7 of the Algal Assay Procedure: Bottle Test (E.P.A., 1971).

Physical and chemical data from the Des Moines River Quality Network Study (Baumann et al., 1980) conducted by the Iowa State Engineering Institute were utilized and correlated where appropriate.

RESULTS

Table 3 summarizes the results obtained from the use of the analysis of variance (E.P.A., 1971) performed to determine the effects of the nutrient spikes on the river water. Actual data and analyses from this study can be found in Appendix I.

Results from the winter 1978 samples showed increases in algal growth significant at the 99% level occurring from the addition of macronutrients alone at Station 1, and at Stations 1, 2 and 3 from the addition of trace metals in combination with iron and manganese. Growth increases, significant at the 95% level, occurred at Station 1 with the addition of phosphorus and nitrogen in combination with macronutrients, and with iron and manganese, phosphorus and nitrogen and macronutrients in combination. One decrease in algal growth, significant at the 95% level, occurred at Station 2 with the addition of trace metals,

Table 2. Design for 2^4 factorial experiments to determine limiting nutrients (E.P.A., 1971).

FACTORS

30% * Macronutrients added (Ca^{++}, Mg^{++}, Cl^{-}, Na^{+}, S^{++})	+								-							
30% * P & N added	+				-				+				-			
30% * Fe & Mn added	+		-		+		-		+		-		+		-	
30% * other trace metals added (B^{+3}, Zn^{++}, Co^{++}, Cu^{++}, Mo^{++})	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Rep I Flask No.	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31
Rep II Flask No.	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
"Factor" in print out	15	13	14	10	12	9	7	4	11	8	6	3	5	2	1	

* 30% of the reference medium concentration (E.P.A., 1971)
(+) - Factor added
(-) - Factor not added

Table 3. Results from analysis of variance of seasonal samples, indicating statistically significant results from nutrient spike additions.

	WINTER				SPRING				SUMMER				FALL			
Stations:	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
FACTOR																
1. Trace																
2. Fe+Mn																
3. P+N					+		+									
4. Macro	++				++											
5. Trace Fe+Mn	++	++	++						++	++	++	++	++			++
6. Trace P+N						++				++	+		++	+		++
7. Trace Macro																
8. Fe+Mn P+N					--											
9. Fe+Mn Macro									+	+						
10. P+N Macro	+					++										
11. Trace Fe+Mn P+N										+						
12. Trace Fe+Mn Macro		-			+				++	++						
13. Fe+Mn P+N Macro	+				-	-		-								

Table 3 continued.

	WINTER				SPRING				SUMMER				FALL			
Stations:	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
FACTOR																
14. Trace P+N Macro							-	-								
15. Trace Fe+Mn P+N Macro	-														•	

++ indicates significance at the 99% level for increasing algal growth

\+ indicates significance at the 95% level for increasing algal growth

-- indicates significance at the 99% level for decreasing algal growth

\- indicates significance at the 95% level for decreasing algal growth

iron and manganese and macronutrients in combination.

Spring 1979 results showed increases in algal growth significant at the 99% level occurring at Station 1 with the addition of macronutrients alone, at Station 2 with the addition of trace metals in combination with phosphorus and nitrogen, and at Station 2 with the addition of macronutrients in combination with phosphorus and nitrogen. Increases in growth, significant at the 95% level, occurred at Stations 1 and 3 with the addition of phosphorus and nitrogen, and at Station 1 with the addition of trace metals in combination with iron and manganese and macronutrients. A decrease in growth, significant at the 99% level, occurred at Station 1 with the addition of iron and manganese in combination with phosphorus and nitrogen. Decreases in algal growth, significant at the 95% level, occurred at Stations 1, 2 and 4 with the addition of iron and manganese in combination with phosphorus and nitrogen and macronutrients, and at Station 3 with the addition of trace metals, phosphorus and nitrogen and macronutrients in combination.

Results from the summer 1979 samples showed increases in growth significant at the 99% level occurring at Stations 1, 2, 3 and 4 with the addition of trace metals in combination with iron and manganese, at Station 2 with the addition of trace metals in combination with phosphorus and nitrogen, and at Stations 1 and 2 with the addition of trace metals,

iron and manganese and macronutrients in combination. Increases in growth, significant at the 95% level, occurred at Station 3 with the addition of trace metals in combination with phosphorus and nitrogen, at Stations 1 and 2 with the addition of iron and manganese in combination with macronutrients, and at Station 2 with the addition of trace metals, iron and manganese, and phosphorus and nitrogen in combination. No decreases in algal growth significant at the 99% or 95% levels occurred at any station in the summer 1979 sample.

Fall 1979 results showed increases in algal growth significant at the 99% level occurring at Stations 1 and 4 with the addition of trace metals in combination with iron and manganese, and at Stations 1 and 4 with the addition of trace metals in combination with phosphorus and nitrogen. One increase in growth, significant at the 95% level, occurred at Station 2 with the addition of trace metals in combination with phosphorus and nitrogen. Again, no significant decreases in growth occurred at any station in the fall 1979 sample.

DISCUSSION

Selenastrum capricornutum has been found to have several superior qualities as a laboratory test organism: (1) it is solitary; (2) it is easy to identify; (3) it grows easily in culture in a variety of different test waters with

little variation in form; (4) it can tolerate both strongly acidic and alkaline waters; and (5) it is an obligate autotroph (Forsberg, 1972; Reynolds et al., 1975). For these reasons, S. capricornutum has been chosen for use in the Algal Assay Procedure: Bottle Test by E.P.A., and was so used in this study.

Schelske et al. (1978) used algal assays with existing or natural phytoplankton assemblages in the Laurentian Great Lakes for two reasons: (1) these organisms would respond to artificial or natural enrichment in the lake ecosystem; and (2) natural phytoplankton have a physiological history under the influence of environmental conditions in the lake and consequently would express responses to nutrient enrichment that are not readily predicted.

In their study concerning a proposed cooling lake in Kansas, Gerhold and Otto (1976) found that the genus S. capricornutum was a component of the ecosystem, but felt that it need not have occurred naturally in the lake or streams involved to be justified as a test species in the laboratory. The objective of their procedure was to determine the total quantity of algal biomass supportable by the test waters, not the ecological relationships of the native species. Because all algae require approximately the same nutrients to build protoplasm, all that is required of a test species in conducting eutrophication assessment bioassays is that it

be easily handled in the laboratory and that its general physiology be reasonably representative. S. capricornutum serves well in meeting the objectives in this type of monitoring program.

The recommended incubation conditions for S. capricornutum are 24 ± 2°C under continuous cool-white Fluorescent lighting at 4304 lumens (400 ± 10% foot-candles) (E.P.A., 1971). The incubation conditions used in this study were modified somewhat. The incubator used was determined to have 450 foot-candles of illumination, as mentioned previously, and the temperature inside the incubator was 23°C. Due to limitations of the incubator, the illumination period was not continuous but was for 18 hours, followed by 6 hours of darkness. It is unlikely that these modifications significantly altered the results obtained in this study.

The recommended water sample pretreatment method (E.P.A., 1971 and Miller et al., 1978) calls for autoclaving followed by filtration in order to remove indigenous organisms and suspended solids. Various opinions have developed as some experimenters prefer one pretreatment method over another.

Jadlocki et al. (1976) studied the effects of water hardness, phosphorus concentration and sample pretreatment on the Algal Assay Procedure: Bottle Test. Algal assays were done on water samples before treatment or after

pretreatment by autoclaving and/or filtration. The results indicated that pretreatment of the water samples may cause erratic results. The controlling factors include water hardness, phosphorus concentration, and standing algal crops. Waters which are very hard (above 200 ppm as calcium carbonate) and have a very high orthophosphate content (above 10 µg/l) may form a precipitate during autoclaving which effectively depletes the available phosphorus, especially if the precipitate is removed by filtration. A substantial growth of indigenous algae (over 200 fluorescence units from *in vivo* Chlorophyll *a*) in these waters would release enough dissolved phosphorus during autoclaving to cause a net increase in soluble phosphorus concentration. Filtration pretreatment may also remove large amounts of total phosphorus from eutrophic water samples.

Filip and Middlebrooks (1975) evaluated sample preparation techniques for algal bioassays by examining the chemical and biostimulatory properties of mesotrophic pond water and eutrophic feedlot run-off after subsamples of each were separately subjected to standard membrane filtration and autoclaving pretreatments for bottle test bioassays. These pretreatments fulfilled the requirement of destroying or removing indigenous algae, but each technique substantially altered the water chemistry and changed bioassay results. Filtration removed phosphorus; autoclaving raised the pH value, precipitated salts and eliminated carbon

dioxide.

Algal assay tests of water samples from four lakes in the Madison, Wisconsin area after membrane filtration of the samples, indicated all four of the test waters were deficient in iron; however there was no response to added iron when autoclaved samples were tested. Thus, these water samples must have ordinarily contained adequate available iron which was removed by filtration. Available phosphorus, nitrogen and iron can be released from in situ algae in autoclaved samples (Fitzgerald, 1975).

For this study, it is believed that autoclaving alone was sufficient as a pretreatment method for the Des Moines River water samples as filtration might have removed excess amounts of nutrients, and it was desired to assess the total algal potential which could develop from all possible nutrient sources in the river water.

Saldick and Jadlocki (1978) studied sample pretreatment methods before inoculation with test algae in algal assays. It was found that the nutritional state of the algae present in the Bottle Test samples influenced the test results and must be considered in their interpretation, inferring that if inoculated algae are to be test organisms, it might be best to "starve" the algal cells before inoculating them into the test water.

Keenan and Auer (1974) examined the effects of luxury uptake (storage of excess nutrient beyond physiological

needs) of phosphorus on the sensitivity of algal bioassays. In most cases, the algal growth response is limited by some nutrient, and the bioassay procedure should be capable of indicating differences in the concentration of the nutrient. The bioassay will lack sensitivity if the test strain of algae has an extraneous source of the limiting nutrient. Stored phosphorus could act as an extraneous source of phosphorus in situations where that nutrient is scarce. The potential loss of sensitivity resulting from nutrient carryover is recognized in the algal assay procedure, but the possible importance of luxury uptake is not stressed. It was found that the influence of luxury uptake of phosphorus on algal bioassays is a function of the phosphorus concentration in the original stock culture growth medium and of the length of time during which the cells are starved of phosphorus.

It is not likely that luxury uptake of phosphorus from the original stock culture medium could have any significant influence in the results obtained here as the river water sampled was not deficient in phosphorus (I.D.E.Q., 1975).

Chiudani and Vighi (1974) found the nitrogen:phosphorus (N:P) ratio for the physiological requirements for Selenastrum capricornutum to be 10:1. Miller et al. (1976a) determined the critical N:P ratio necessary for maximum yield of S. capricornutum in a test water to be 11.3 µg N:

1 µg P. The N:P ratio was found to be useful in preliminary assessment of algal growth limitation in natural waters. Waters containing N:P ratios less than 10 may be considered nitrogen limiting while those waters with N:P ratios greater than 10 may be phosphorus limiting for algal growth.

Although it is unlikely that the Des Moines River is limiting in phosphorus or nitrogen for algal growth, when comparing N:P ratios for the Des Moines River, phosphorus appears to be limiting (N:P ratios greater than 10) for most of the year at most of the sampling stations (Table 4). Nitrogen appears to be limiting (N:P ratios less than 10) at Station 3 in the summer and fall.

Shiroyama et al. (1975) developed factors for ascertaining the maximum yield of S. capricornutum for orthophosphate and total soluble inorganic nitrogen (NO_2^-,NO_3^-,NH_3) in the presence of all other essential nutrients. When other nutrients are not growth-limiting, the addition of 0.001 mg orthophosphate per liter to water containing > 0.010 mg/l orthophosphate will produce 0.43 mg dry weight/l of S. capricornutum. Therefore, the potential dry weight yield, in mg/l, of a water can be obtained by multiplying the mg/l orthophosphate by 430. In cases where nitrogen is the growth-limiting nutrient, total soluble inorganic nitrogen can be converted to maximum yield of S. capricornutum by multiplying the total inorganic N (mg/l) by a factor of 38. Each milligram of total soluble inorganic N will produce

Table 4. Orthophosphate and total soluble inorganic nitrogen (TSIN) levels in Des Moines River water samples (Baumann et al., 1980), corresponding predicted algal yield levels, actual algal yield levels from control flasks, and N:P ratios.

SEASON	STATION	Ortho-P (mg/l)	Predicted Yield-mg/l ortho-P x 430	TSIN (mg/l)	Predicted Yield-mg/l TSIN x 38
Winter	1	0.46	200	9.10	345.6
	2	0.04	20	6.98	265.0
	3	0.78	330	9.64	366.5
	4	0.44	190	7.50	285.2
Spring	1	0.16	70	10.34	393.1
	2	0.20	90	9.94	377.7
	3	0.29	120	11.98	455.0
	4	0.34	150	8.91	338.6
Summer	1	0.51	220	5.80	220.4
	2	0.58	250	6.19	235.2
	3	0.66	280	6.31	239.8
	4	0.58	250	6.64	252.3
Fall	1	0.34	150	5.50	209.0
	2	0.37	160	4.96	188.4
	3	0.79	340	5.14	195.3
	4	0.51	220	5.00	190.0

Table 4. continued

SEASON	STATION	Actual Average Yield of Two Control Flasks (mg/l)	N:P Ratio
Winter	1	173.7	20
	2	118.7	175
	3	143.7	12
	4	187.3	17
Spring	1	238.3	65
	2	117.7	50
	3	244.7	41
	4	112.3	26
Summer	1	280.7	11
	2	184.3	11
	3	185.7	9.6
	4	214.0	11
Fall	1	182.0	16
	2	210.3	13
	3	246.0	6.5
	4	236.3	9.8

38 mg dry weight of S. capricornutum per liter.

Taking into consideration such factors as chemical analysis, procedural errors during test preparation and sampling, and environmental fluctuations within the culture chambers, yields falling within ± 20% of the computed maximum yields are considered statistically valid (Shiroyama et al., 1975). Furthermore, the productivity values reflect the river waters' algal growth potential only at the time of assay.

As the N:P ratios from the Des Moines River samples were greater than 10 for most sampling periods, indicating phosphorus limitation, predictive yields using orthophosphate levels were compared to actual yield from the assay control flasks (Table 4). Actual yield for Station 1 above Saylorville Reservoir falls within the ± 20% limitation of the predicted yield (indicating that the predicted growth levels were obtained) during the winter only. Actual yield greatly exceeded predicted yield during the spring, summer and fall. Algal assay results indicate that addition of nutrient spikes caused increases in growth at Station 1 most frequently in the winter and least frequently in the fall, possibly indicating that nutrients were limiting in the winter, but not during the remaining seasons of the year. It is necessary to note that as water samples were not filtered, some of the dry weight obtained for each assay must be attributed to suspended solids in the water.

Predicted yield as compared to actual yield is only an estimate, then, of the algal growth potential in the Des Moines River. If actual yield fell short of the predicted yield, and some of the actual yield is suspended solid, it can then be assumed that the actual yield did indeed fall short of the predicted yield. Other comparisons would not be as clear, especially as actual yield came close to predicted yield, since the amount of suspended solid in each dry weight sample is not known.

The actual yield obtained from the water samples taken from Station 2, below Saylorville Dam and above the City of Des Moines, greatly exceeded the predicted yield in the winter, spring and fall, but not in the summer. Actual yield in the summer was much less than the predicted yield. Also, growth increases obtained from nutrient additions in algal assay were more frequent in the summer, possibly indicating that some nutrient limitations were present at Station 2 during the summer.

Actual yield for Station 3, below Des Moines and above Red Rock Reservoir, can be compared with predicted levels of algal growth using orthophosphate content during the winter and spring, but must be compared with predicted levels of growth using nitrogen content in the summer and fall as the N:P ratio was less than 10 during those seasons, indicating nitrogen-limited conditions. At Station 3 in the winter, predicted yield greatly exceeded the actual

yield, yet in the spring, actual yield exceeded predicted yield. Nutrient additions in algal assay to waters collected in the spring did little to increase growth, indicating that sufficient nutrients were present at that time. Using nitrogen content, the actual yield fell short of the predicted yield in the summer, but slightly exceeded the predicted yield in the fall. Assay results showed that growth increases as a result of nutrient additions at Station 3 were more common in the summer and least common in the fall, indicating that nutrients were limiting in the summer but were present in sufficient quantities in the fall.

At Station 4, below Red Rock Dam, actual yield fell short of the predicted yield only during the spring; the results from the other three sampling periods showed that actual yield fell within ± 20% of the predicted yield. Assay results showed that nutrient additions to samples from Station 4 increased growth in the fall and summer infrequently, and did not increase growth in the winter and spring. Since actual yield in the spring fell short of the predicted yield, and nutrient additions did not increase algal growth, some toxic materials that would have inhibited growth might have been present.

The presence of Saylorville and Red Rock Reservoirs likely affects the potential algal productivity in the downstream water, since it has been shown to affect the nutrient levels occurring in the Des Moines River (Baumann

et al., 1980). A reservoir represents a special case in terms of water quality: sediment transport is a dominant process in reservoirs, therefore they are not like lakes, but settling-out does occur due to decreased flow rate, hence reservoirs are not like rivers. Using this reasoning, differences in algal growth potential above and below reservoirs might logically be expected.

However, in this study, above- and below-reservoir differences are not distinct. Instead, addition of nutrient spikes to test waters more frequently stimulated growth at Stations 1 and 2 above the City of Des Moines than at Stations 3 and 4 below Des Moines. Very few nutrient spikes affected the algal growth, either by causing increases or decreases, in the waters collected from Station 3 immediately below Des Moines, perhaps because effluent from the Des Moines Wastewater Treatment Plant enters the river three river miles upstream from Station 3. This effluent possibly contains enough nutrients or enough toxic materials that nutrient additions to the river water at this point have little effect. The Raccoon River, another nutrient-laden prairie river, joins with the Des Moines River about seven river miles upstream from Station 3, which likely exerts some effect on the results obtained at both Stations 3 and 4.

Condit (1972) used algal assays to assess algal growth in the Spokane River above and below the City of

Spokane. The growth parameters obtained from control flasks in his study indicated that during the test periods, the total productivity and algal growth rates in the natural waters increased as the river progressed through the city. During the spring, high runoff provided adequate nutrients to promote algal growth in bloom proportions. The large biomass reduced phosphorus concentrations to limiting levels in the river. The especially high algal production in the lower river was due in part to the added phosphorus made by the wastewater treatment plant. Dissolved orthophosphate levels at this station were sufficiently high to cause inhibition to algal growth rates when additional phosphorus was introduced in the bioassay.

In reviewing the algal assay results from Stations 1 and 2 alone, nutrient additions more frequently stimulated growth at the below-reservoir Station 2, indicating that the reservoir acted to settle-out some of the nutrients that were suspended in the water. This same effect, of increased growth with the addition of nutrient spikes at the below-dam site, was not seen at Stations 3 and 4 below Des Moines. It appears likely, then, that the City of Des Moines exerts some effect on the Des Moines River that is not sufficiently altered by Red Rock Reservoir in order to show the changes in algal growth potential that were seen at Stations 1 and 2 above and below Saylorville Reservoir.

Addition of nutrient spikes in various combinations

did not consistently increase growth at the stations along the river. The addition of trace metals in combination with iron and manganese most frequently increased growth followed by trace metals in combination with phosphorus and nitrogen. Other nutrient additions, either alone or in combination, increased growth less frequently. Trace metals added alone caused no significant increases or decreases in algal growth, as was the case with the addition of iron and manganese alone.

Rodhe (1978) suggested that the growth effect of each nutrient is interrelated with the combined effects of all other factors operating within the entire complex of conditions. This means that the growth response of an alga to the most limiting nutrient, say phosphorus, is dependent not only on the supply of phosphorus, but also upon the nutrient levels of other factors that are operationally significant.

It would be logical, therefore, to note that combinations of nutrients, such as trace metals with iron and manganese, are growth-limiting in the Des Moines River while those nutrients alone are not.

Since the predicted yield of S. capricornutum was not attained in the Des Moines River sample waters at Station 2 in the summer, at Station 3 in the winter and summer, and at Station 4 in the spring, it is possible that one of the following might have occurred: (1) there exists

the presence of other growth-limiting nutrients; (2) there exists the presence of toxicants such as heavy metals; and (3) the chemical analyses for N and P might not have been reliable.

In order to assess the effect of the heavy metal zinc on S. capricornutum, Greene et al. (1975b) looked at the toxicity of zinc to S. capricornutum as a function of phosphorus or ionic strength. Their study was concerned with evaluating the ability of the Algal Assay Procedure: Bottle Test to identify the potential toxicity of heavy metals in a defined inorganic medium. Heavy metals are normally found in the environment and perform some critical biologic functions. In excess, however, these substances can exert either acute or chronic toxicity. The state of chemical combination of a pollutant determines its chemical behavior in the aquatic environment. Ionic species, such as heavy metals, tend to be held either in solution or establish ionic exchange equilibria with suspended materials. The introduction of heavy metals into receiving waters and their effects on the biota depend upon a variety of complex responses governed by several basic factors: (1) nature of the metal; (2) heavy metal concentration; (3) characteristics of the receiving system; (4) presence of other toxicants; and (5) exposure time.

Stress to biological populations in multiple use river systems is usually exerted by complex wastes of organic

and inorganic origin. The interactions of these wastes on the growth of planktonic algae in multiple-use river systems has not been defined. The response of an organism to a heavy metal pollutant depends on several basic factors: (1) solubility or ion exchange equilibria; (2) ionic strength; (3) metal concentration; (4) contact time; (5) environmental characteristics of the test; and (6) physiological condition of the test organism.

Chiudani and Vighi (1978) ranked heavy metals according to their increasing toxic effect to Selenastrum capricornutum in the following order: zinc < cadmium < chromium < cobalt < nickel < copper.

Bartlett et al. (1974) found 0.7 mg/l zinc to be algicidal and 0.1 mg/l of zinc to inhibit the growth of Selenastrum.

Historic data (University Hygienic Laboratory, 1980) indicate that at stations closely equivalent to Stations 2 and 3 on the Des Moines River that a minimum of 0.01 mg/l of zinc exists in the river at these stations throughout the year. This amount might likely be present at equal or possibly greater concentrations at the other two stations. However, this amount of zinc has not been determined to be inhibitory to the growth of Selenastrum; therefore, zinc likely does not inhibit algal growth in the Des Moines River.

Baumann et al. (1980) measured total manganese

levels at Station 4 in the Des Moines River to be as high as 0.13 mg/l in the winter months and as low as 0.02 mg/l in the spring. At these levels, it is possible that manganese is inhibitory to S. capricornutum in the Des Moines River, since a 50% reduction in the total algal cell volume of S. capricornutum in standard algal medium was found to have occurred with the addition of 0.0031 mg/l manganese (Christensen and Scherfig, 1979).

Total lead and soluble lead levels were measured to be 0.02 mg/l each on the average at all four stations on the Des Moines River (Baumann et al., 1980). These levels are not likely high enough to be inhibitory to algal growth (Christensen and Scherfig, 1979).

Copper was measured at Stations 2 and 3 to be less than 0.01 mg/l (University Hygienic Laboratory, 1980) and is also not likely to be growth inhibiting in the Des Moines River (Christensen and Scherfig, 1979).

Miller et al. (1976b) used the Algal Assay Procedure: Bottle Test to define the effects of heavy metal stress upon algal growth in natural waters. Since metals are bonded by ligands and particles to varying degrees, the absolute metal concentrations contained in wastewater effluent or receiving waters do not necessarily reflect the degree to which they affect biological systems. In their study, the researchers used EDTA to release iron and manganese naturally present in water and stimulated growth. The concentration of total

soluble ferric ion that can be in equilibrium with ferric hydroxide at pH 8.0 in oxygenated water is approximately 0.2 µg/l. The iron requirement for S. capricornutum is 4.5 µg/l which is 22.5 times greater than the normal concentration in soluble form. Addition of EDTA stabilizes soluble iron availability in natural waters. Organic ligands, such as EDTA, can stabilize ferrous iron through the formation of organic complexes which are resistant to oxygenation in natural waters, thus increasing the availability of iron for biological assimilation.

Miller et al. (1975) and Miller et al. (1976a) used EDTA prior to assay of test waters to remove heavy metal toxicity. EDTA was not used in this study to stabilize the heavy metals present in the river water samples. The procedure for the Selenastrum capricornutum Algal Assay Procedure: Bottle Test (Miller et al., 1978) does not specify its use.

Algal bioassays are quite valuable since they relate biological responses directly to water and its nutrient content. Compared to chemical analyses, algal assays provide more knowledge of the bio-stimulative (or toxic) effects of such waters (Gargas, 1978).

Fitzgerald (1975) stated that evaluations of the Algal Assay Procedure: Bottle Test have demonstrated its value for determining the level of nutrients in water supplies which are available for the growth of algae as

contrasted by chemical analyses of total nutrient contents. The maximum yield is not affected by the supply of carbon in normal algal assay cultures.

As Forsberg (1972) pointed out, if algal assays are to become routine practice, it will be necessary to develop the use of small samples, small culture volumes and short incubation periods. Algal studies normally include a two-week incubation period which is too long for large scale routine testing.

In adapting the Algal Assay Procedure: Bottle Test for further use on the Des Moines River, use of more individual nutrient spikes (such as adding nitrogen alone rather than in combination with phosphorus as a single spike) would be helpful to more closely define nutrient limitations. Increasing the number of sampling sites along with the frequency of sampling, and adding a few sites along the Raccoon River might give added insight to where more distinct changes occur in the algal productivity of the Des Moines River. Use of EDTA to chelate heavy metals prior to assay could be recommended.

It has been shown that addition of certain nutrient combinations increased growth in the Des Moines River samples. This algal growth was most often increased with the addition of trace metals in combination with iron and manganese, and trace metals in combination with phosphorus and nitrogen. Other nutrient spikes increased growth with

less regularity. Actual algal yield correlated with predicted yield of S. capricornutum in most of the samples, with exceptions at Station 2 in the summer, Station 3 in the winter and summer and Station 4 in the spring, when actual yield fell short of predicted yield. Differences in algal growth potential at the above- and below-reservoir stations were not distinct. At Station 2 below Saylorville Reservoir, nutrient additions more frequently increased growth than at Station 1 above Saylorville. The same effect was not seen at Stations 3 and 4 above and below Red Rock Reservoir, below the City of Des Moines.

SUMMARY

1. Selenastrum capricornutum was grown in an algal assay which combined various combinations of nutrient spikes to Des Moines River water above and below Saylorville and Red Rock Reservoirs. Growth response was measured gravimetrically.
2. Seasonal river samples were taken from December 1978 until October 1979. Nutrient levels were obtained from Baumann et al. (1980) and University Hygienic Laboratory (1980).
3. Results show that trace metals in combination with iron and manganese increased growth more frequently along the Des Moines River, followed by trace metals in combination with phosphorus and nitrogen.

4. Actual yield correlated with predicted algal yields of S. capricornutum using phosphorus and nitrogen content of the waters, with exceptions at Station 2 in the summer, Station 3 in the winter and summer, and Station 4 in the spring.
5. Using N:P ratios, phosphorus is most often the limiting factor in the Des Moines River, although addition of N and P in assay rarely increased growth.
6. Above- and below-reservoir differences in algal productivity were not distinct. Samples taken from the station below Saylorville responded more frequently to nutrient additions than did samples taken from the corresponding above-reservoir station. Differences above and below Red Rock Reservoir (below the City of Des Moines) were less distinct.
7. The possibility exists that heavy metals may inhibit algal growth in the Des Moines River during certain times of the year, as seen by the presence of high levels of manganese.
8. Further study should include a year-round survey using more sampling sites and frequent collections to determine the algal growth potential and serve as a basis for predicting future trends.

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APPENDIX

APPENDIX

Tables A through P summarize the analysis of variance of the 2^4 factorial design (E.P.A., 1971) performed to determine the effects of the nutrient spikes.

Factor refers to the elements added as shown in Table 3. Each factor line shown in Tables A through P refers to a statistical comparison between those flasks which had the element added versus those flasks which did not have the same element added.

Comparison refers to the effect of addition of the groups of nutrient spikes, where the sign indicates the direction of effect of addition. A negative sign means that addition of that particular combination of factors resulted in a decrease of growth.

F-Ratio indicates significance at either the 99% or the 95% level of the effect of the nutrient addition for each factor.

Mean 1 indicates the mean of the flasks with the element added; Mean 2 indicates the mean of the flasks without the element added.

Table A. Sample data and analysis of variance of winter sample, Station 1.

SAMPLES		SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1	2	4.02, 3.71	7.73	3.86
3	4	3.23, 3.71	6.94	3.47
5	6	3.19, 3.28	6.47	3.23
7	8	3.38, 3.11	6.49	3.24
9	10	3.11, 2.35	5.46	2.73
11	12	2.88, 3.40	6.28	3.14
13	14	2.68, 2.51	5.19	2.59
15	16	2.60, 2.47	5.07	2.53
17	18	4.16, 3.86	8.02	4.01
19	20	3.33, 3.52	6.85	3.42
21	22	2.78, 2.77	5.55	2.77
23	24	3.16, 3.39	6.55	3.27
25	26	2.28, 2.36	4.64	2.32
27	28	2.23, 2.59	4.82	2.41
29	30	2.63, 2.61	5.24	2.62
31	32	2.32, 2.89	5.21	2.60

Mean square (between samples): 0.53
Mean square (within samples): 6.07 E-2
Mean square (replicates): 9.45 E-3
Error: 6.41 E-2

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.26	3.36 E-2	6.03	0
2	0.31	0.05	6.03	0
3	0.09	3.95 E-3	3.02	3.01
4	4.97	**12.0	3.17	2.86
5	12.6	**78.5	3.41	2.62
6	2.75	3.69	3.10	2.93
7	1.83	1.63	3.07	2.96
8	1.79	1.56	3.07	2.96
9	0.05	1.22 E-3	3.02	3.01
10	3.99	*7.76	3.14	2.89
11	1.41	0.97	3.06	2.97
12	-1.43	0.99	2.97	3.06
13	4.13	*8.32	3.14	2.89
14	-2.09	2.13	2.95	3.08
15	-3.53	*6.08	2.91	3.13

** - significant at 99% level
* - significant at 95% level

Table B. Sample data and analysis of variance of winter sample, Station 2.

SAMPLES		SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1	2	2.13, 2.44	4.57	2.28
3	4	1.79, 2.19	3.98	1.99
5	6	2.32, 2.05	4.37	2.18
7	8	2.39, 2.03	4.42	2.21
9	10	1.77, 1.64	3.41	1.70
11	12	1.63, 1.61	3.24	1.62
13	14	2.05, 1.76	3.81	1.90
15	16	1.58, 1.73	3.31	1.65
17	18	2.31, 2.28	4.59	2.29
19	20	2.55, 2.21	4.76	2.38
21	22	2.34, 2.63	4.97	2.48
23	24	2.35, 2.09	4.44	2.22
25	26	1.49, 1.34	2.83	1.41
27	28	1.46, 1.70	3.16	1.58
29	30	1.66, 1.69	3.35	1.67
31	32	1.74, 1.82	3.56	1.78

Mean square (between samples): 0.22
Mean square (within samples): 2.93 E-2
Mean square (replicates): 3.83 E-3
Error: 3.10 E-2

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.17	2.93 E-2	3.92	0
2	0.20	4.26 E-2	3.92	0
3	1.03	1.07	1.99	1.93
4	-1.69	2.87	1.91	2.01
5	9.43	**89.6	2.26	1.67
6	-0.55	0.30	1.94	1.98
7	-0.51	0.26	1.94	1.97
8	0.77	0.60	1.98	1.94
9	1.39	1.95	2.00	1.92
10	1.09	1.20	1.99	1.93
11	0.27	7.34 E-2	1.97	1.95
12	-2.29	*5.28	1.89	2.03
13	0.39	0.15	1.97	1.95
14	1.13	1.29	1.99	1.93
15	-0.63	0.40	1.94	1.98

** - significant at 99% level
* - significant at 95% level

Table C. Sample data and analysis of variance of winter sample, Station 3.

SAMPLES		SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1	2	3.04, 2.68	5.72	2.86
3	4	2.91, 2.82	5.73	2.86
5	6	2.85, 3.25	6.10	3.05
7	8	2.55, 3.12	5.67	2.83
9	10	2.53, 2.22	4.75	2.37
11	12	2.35, 2.78	5.13	2.56
13	14	3.02, 2.28	5.30	2.65
15	16	2.27, 2.13	4.40	2.20
17	18	3.12, 2.60	5.72	2.86
19	20	2.93, 2.41	5.34	2.67
21	22	2.74, 2.56	5.30	2.65
23	24	2.67, 2.29	4.96	2.48
25	26	1.86, 2.55	4.41	2.20
27	28	2.31, 2.42	4.73	2.36
29	30	2.26, 2.29	4.55	2.27
31	32	2.35, 1.96	4.31	2.15

Mean square (between samples): 0.16
Mean square (within samples): 0.09
Mean square (replicates): 0.06
Error: 9.02 E-2

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.22	1.72 E-2	5.13	0
2	0.27	2.53 E-2	5.13	0
3	1.58	0.86	2.62	2.52
4	0.94	0.31	2.50	2.54
5	6.96	**16.8	2.78	2.35
6	3.48	4.19	2.68	2.46
7	-2.24	1.74	2.60	2.64
8	0.70	0.17	2.59	2.54
9	0.30	0.03	2.58	2.56
10	0.02	1.38	2.57	2.56
11	-1.22	0.52	2.53	2.60
12	0.32	3.55 E-2	2.58	2.56
13	1.44	0.72	2.61	2.52
14	-1.20	0.50	2.53	2.60
15	-1.02	0.36	2.53	2.60

** - significant at 99% level
* - significant at 95% level

Table D. Sample data and analysis of variance of winter sample, Station 4.

SAMPLES		SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1	2	3.20, 3.46	6.66	3.33
3	4	3.19, 3.10	6.29	3.14
5	6	2.19, 3.01	5.20	2.60
7	8	2.84, 3.50	6.34	3.17
9	10	3.00, 2.91	5.91	2.95
11	12	2.93, 3.07	6.00	3.00
13	14	2.79, 2.79	5.58	2.79
15	16	2.72, 2.74	5.46	2.73
17	18	2.69, 3.40	6.09	3.04
19	20	2.75, 2.73	5.48	2.74
21	22	2.74, 2.99	5.73	2.86
23	24	3.00, 3.26	6.26	3.13
25	26	2.81, 2.92	5.73	2.86
27	28	3.32, 2.61	5.93	2.96
29	30	2.72, 2.49	5.21	2.60
31	32	2.60, 3.02	5.62	2.81

Mean square (between samples): 8.74 E-2
Mean square (within samples): 8.10 E-2
Mean square (replicates): 0.20
Error: 7.33 E-2

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.26	2.80 E-2	5.84	0
2	0.31	4.05 E-2	5.84	0
3	-1.27	0.69	2.88	2.96
4	2.69	3.09	3.00	2.84
5	2.61	2.90	3.00	2.84
6	1.39	0.82	2.96	2.88
7	2.65	2.99	3.00	2.84
8	-0.11	5.16 E-3	2.92	2.92
9	-0.21	1.88 E-2	2.92	2.93
10	-0.71	0.21	2.90	2.94
11	1.87	1.49	2.98	2.86
12	0.47	0.09	2.94	2.91
13	2.65	2.99	3.00	2.84
14	-0.05	1.07	2.92	2.92
15	1.79	1.37	2.98	2.86

** - significant at 99% level
* - significant at 95% level

Table E. Sample data and analysis of variance of spring sample, Station 1.

SAMPLES	SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1 2	3.85, 3.84	7.69	3.84
3 4	4.58, 4.03	8.61	4.30
5 6	3.12, 3.16	6.28	3.14
7 8	3.69, 2.86	6.55	3.27
9 10	3.02, 5.10	8.12	4.06
11 12	3.35, 3.12	6.47	3.23
13 14	3.62, 3.81	7.43	3.71
15 16	2.73, 3.29	6.02	3.01
17 18	3.83, 2.99	6.82	3.41
19 20	3.76, 3.90	7.66	3.83
21 22	3.57, 3.34	6.91	3.45
23 24	2.94, 3.83	6.77	3.38
25 26	6.52, 5.91	12.4	6.21
27 28	3.26, 3.13	6.39	3.19
29 30	4.24, 3.80	8.04	4.02
31 32	3.31, 3.84	7.15	3.57

Mean square (between samples): 1.15
Mean square (within samples): 0.25
Mean square (replicates): 0.01
Error: 0.27

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.33	1.24 E-2	7.46	0
2	0.40	1.81 E-2	7.46	0
3	8.10	*7.56	3.98	3.48
4	9.04	**9.41	4.01	3.45
5	-4.76	2.61	3.58	3.88
6	-5.0	2.88	3.57	3.88
7	3.76	1.63	3.85	3.61
8	-11.9	**16.2	3.36	4.10
9	-4.36	2.19	3.59	3.86
10	-0.5	2.88 E-2	3.71	3.74
11	0.18	3.73 E-3	3.74	3.72
12	6.94	*5.55	3.95	3.51
13	-7.02	*5.68	3.51	3.95
14	-4.58	2.42	3.59	3.87
15	5.16	3.07	3.89	3.57

** - significant at 99% level
* - significant at 95% level

Table F. Sample data and analysis of variance of spring sample, Station 2.

SAMPLES		SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1	2	1.87, 2.12	3.99	1.99
3	4	2.50, 2.40	4.90	2.45
5	6	1.66, 1.46	3.12	1.56
7	8	1.63, 1.25	2.88	1.44
9	10	1.67, 1.80	3.47	1.73
11	12	1.85, 1.56	3.41	1.70
13	14	1.44, 1.91	3.35	1.67
15	16	2.43, 2.19	4.62	2.31
17	18	1.20, 1.21	2.41	1.20
19	20	2.40, 1.02	3.42	1.71
21	22	1.55, 1.44	2.99	1.49
23	24	1.51, 1.29	2.80	1.40
25	26	1.59, 1.19	2.78	1.39
27	28	1.46, 1.34	2.80	1.40
29	30	1.53, 1.87	3.40	1.70
31	32	1.79, 1.74	3.53	1.76

Mean square (between samples): 0.22
Mean square (within samples): 9.04 E-2
Mean square (replicates): 0.16
Error: 8.55 E-2

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.15	8.02 E-3	3.37	0
2	0.18	1.12 E-2	3.37	0
3	-2.85	2.97	1.59	1.77
4	0.49	8.77 E-2	1.70	1.67
5	-0.85	0.26	1.66	1.71
6	5.61	**11.5	1.86	1.51
7	-0.91	0.30	1.66	1.71
8	-0.13	6.17	1.68	1.69
9	-0.91	0.30	1.66	1.71
10	5.37	**10.5	1.85	1.52
11	3.11	3.53	1.78	1.59
12	0.93	0.32	1.71	1.65
13	-3.79	*5.25	1.56	1.80
14	1.27	0.59	1.72	1.64
15	2.59	2.45	1.76	1.60

** - significant at 99% level
* - significant at 95% level

Table G. Sample data and analysis of variance of spring sample, Station 3.

SAMPLES		SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1	2	2.81, 4.14	6.95	3.47
3	4	4.07, 4.74	8.81	4.40
5	6	3.92, 4.35	8.27	4.13
7	8	3.26, 2.37	5.63	2.81
9	10	3.57, 3.81	7.38	3.69
11	12	3.19, 2.59	5.78	2.89
13	14	3.06, 3.78	6.84	3.42
15	16	4.17, 2.94	7.11	3.55
17	18	6.49, 4.40	10.9	5.44
19	20	3.68, 3.17	6.85	3.42
21	22	3.13, 3.88	7.01	3.50
23	24	3.51, 2.76	6.27	3.13
25	26	3.85, 5.13	8.98	4.49
27	28	2.91, 2.67	5.58	2.79
29	30	3.87, 3.55	7.42	3.71
31	32	3.97, 3.37	7.34	3.67

Mean square (between samples): 0.96
Mean square (within samples): 0.42
Mean square (replicates): 0.10
Error: 0.44

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.32	7.15 E-3	7.32	0
2	0.38	1.04 E-2	7.32	0
3	10.4	*7.55	3.98	3.34
4	5.33	1.99	3.83	3.50
5	4.25	1.27	3.79	3.53
6	-3.57	0.89	3.55	3.77
7	3.99	1.12	3.78	3.54
8	0.75	3.95 E-2	3.68	3.64
9	-6.15	2.66	3.47	3.85
10	7.31	3.75	3.89	3.43
11	-3.19	0.71	3.56	3.76
12	0.85	5.07 E-2	3.69	3.63
13	-6.39	2.87	3.46	3.86
14	-9.25	*6.01	3.37	3.95
15	-2.01	0.28	3.60	3.72

** - significant at 99% level
* - significant at 95% level

Table H. Sample data and analysis of variance of spring sample, Station 4.

SAMPLES	SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1 2	1.76, 1.53	3.29	1.64
3 4	2.32, 2.34	4.66	2.33
5 6	2.54, 1.95	4.49	2.24
7 8	1.53, 2.14	3.67	1.83
9 10	1.67, 1.59	3.26	1.63
11 12	1.74, 2.10	3.84	1.92
13 14	1.69, 1.33	3.02	1.51
15 16	1.64, 1.27	2.91	1.45
17 18	1.32, 1.87	3.19	1.59
19 20	1.70, 2.14	3.84	1.92
21 22	1.74, 1.99	3.73	1.86
23 24	2.20, 1.74	3.94	1.97
25 26	2.66, 2.38	5.04	2.52
27 28	1.56, 1.33	2.89	1.44
29 30	1.39, 1.86	3.25	1.62
31 32	1.64, 1.73	3.37	1.68

Mean square (between samples): 0.20
Mean square (within samples): 7.21 E-2
Mean square (replicates): 1.13 E-3
Error: 7.68 E-2

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.16	0.01	3.65	0
2	0.19	1.53 E-2	3.65	0
3	0.15	9.15 E-3	1.83	1.82
4	1.63	1.08	1.88	1.77
5	3.23	4.24	1.92	1.72
6	-0.11	4.92 E-3	1.82	1.83
7	-1.05	0.45	1.79	1.86
8	-2.97	3.59	1.73	1.92
9	-2.19	1.95	1.76	1.89
10	-3.33	4.51	1.72	1.93
11	0.29	3.42 E-2	1.83	1.82
12	2.93	3.49	1.92	1.73
13	-4.21	*7.21	1.69	1.96
14	-4.71	*9.02	1.68	1.97
15	0.57	0.13	1.84	1.81

** - significant at 99% level
* - significant at 95% level

Table I. Sample data and analysis of variance of summer sample, Station 1.

SAMPLES		SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1	2	4.94, 4.89	9.83	4.91
3	4	4.63, 4.99	9.62	4.81
5	6	3.86, 5.20	9.06	4.53
7	8	4.80, 5.14	9.94	4.97
9	10	3.68, 4.12	7.80	3.90
11	12	3.22, 3.67	6.89	3.44
13	14	3.84, 3.99	7.83	3.91
15	16	3.48, 3.83	7.31	3.65
17	18	4.66, 4.26	8.92	4.46
19	20	4.09, 4.55	8.64	4.32
21	22	3.74, 4.25	7.99	3.99
23	24	4.33, 4.99	9.32	4.66
25	26	3.40, 3.86	7.26	3.63
27	28	3.79, 4.88	8.67	4.33
29	30	3.50, 3.88	7.38	3.69
31	32	4.21, 4.21	8.42	4.21

Mean square (between samples): 0.47
Mean square (within samples): 0.16
Mean square (replicates): 1.34
Error: 8.41 E-2

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.37	5.02 E-2	8.43	0
2	0.44	0.07	8.43	0
3	-2.74	2.79	4.13	4.30
4	0.38	5.37 E-2	4.23	4.20
5	11.8	**51.4	4.58	3.85
6	1.68	1.05	4.27	4.16
7	2.72	2.75	4.30	4.13
8	-0.70	0.18	4.19	4.24
9	4.26	*6.74	4.35	4.08
10	1.02	0.39	4.25	4.18
11	-0.38	5.37 E-2	4.20	4.23
12	5.48	**11.2	4.39	4.04
13	2.68	2.66	4.30	4.13
14	0.24	2.14 E-2	4.22	4.21
15	0.78	0.23	4.24	4.19

** - significant at 99% level
* - significant at 95% level

Table J. Sample data and analysis of variance of summer sample, Station 2.

SAMPLES	SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1 2	3.62, 3.34	6.96	3.48
3 4	2.78, 3.43	6.21	3.10
5 6	3.60, 3.11	6.71	3.35
7 8	3.17, 2.59	5.76	2.88
9 10	2.51, 2.71	5.22	2.61
11 12	2.64, 2.54	5.18	2.59
13 14	2.66, 2.14	4.80	2.40
15 16	2.55, 2.39	4.94	2.47
17 18	2.43, 2.37	4.80	2.40
19 20	3.14, 3.01	6.15	3.07
21 22	2.52, 2.73	5.25	2.62
23 24	2.64, 2.67	5.31	2.65
25 26	2.29, 2.20	4.49	2.24
27 28	2.62, 2.15	4.77	2.38
29 30	2.98, 2.55	5.53	2.76
31 32	2.82, 2.71	5.53	2.76

Mean square (between samples): 0.26
Mean square (within samples): 5.98 E-2
Mean square (replicates): 0.17
Error: 5.25 E-2

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.24	0.03	5.48	0
2	0.29	0.05	5.48	0
3	-0.09	4.81 E-3	2.74	2.74
4	-0.05	1.49 E-3	2.74	2.74
5	6.69	**26.6	2.95	2.53
6	3.95	**9.28	2.86	2.61
7	-1.59	1.50	2.69	2.79
8	0.67	0.27	2.76	2.72
9	3.29	*6.44	2.84	2.64
10	2.23	2.96	2.81	2.67
11	2.77	*4.56	2.82	2.65
12	4.31	**11.1	2.87	2.60
13	-1.39	1.15	2.69	2.78
14	1.55	1.43	2.79	2.69
15	-2.15	2.75	2.67	2.81

** - significant at 99% level
* - significant at 95% level

Table K. Sample data and analysis of variance of summer sample, Station 3.

SAMPLES		SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1	2	3.81, 2.58	6.39	3.19
3	4	2.78, 2.97	5.75	2.87
5	6	2.76, 3.79	6.55	3.27
7	8	3.67, 3.71	7.38	3.69
9	10	2.77, 3.10	5.87	2.93
11	12	2.41, 2.97	5.38	2.69
13	14	2.88, 2.80	5.68	2.84
15	16	2.98, 3.06	6.04	3.02
17	18	2.75, 3.05	5.80	2.90
19	20	2.83, 3.33	6.16	3.08
21	22	3.07, 3.21	6.28	3.14
23	24	2.62, 2.87	5.49	2.74
25	26	2.66, 1.97	4.63	2.31
27	28	2.48, 2.68	5.16	2.58
29	30	2.35, 2.33	4.68	2.34
31	32	2.64, 2.93	5.57	2.78

Mean square (between samples): 0.24
Mean square (within samples): 0.13
Mean square (replicates): 0.11
Error: 0.13

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.25	1.56 E-2	5.80	0
2	0.30	2.26 E-2	5.80	0
3	-1.05	0.27	2.87	2.93
4	-2.53	1.56	2.82	2.98
5	6.79	**11.2	3.11	2.69
6	5.27	*6.77	3.06	2.74
7	1.53	0.57	2.95	2.85
8	1.53	0.57	2.95	2.85
9	0.93	0.21	2.93	2.87
10	-0.67	0.11	2.88	2.92
11	-1.99	0.96	2.84	2.96
12	-0.59	8.48 E-2	2.88	2.92
13	-0.89	0.19	2.87	2.93
14	3.11	2.36	3.00	2.80
15	-1.97	0.94	2.84	2.96

** - significant at 99% level
* - significant at 95% level

Table L. Sample data and analysis of variance of summer sample, Station 4.

SAMPLES		SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1	2	4.29, 3.89	8.18	4.09
3	4	2.82, 4.45	7.27	3.63
5	6	3.89, 3.99	7.88	3.94
7	8	4.05, 4.34	8.39	4.19
9	10	3.14, 3.09	6.23	3.11
11	12	3.44, 3.62	7.06	3.53
13	14	3.47, 2.82	6.29	3.14
15	16	3.34, 2.61	5.95	2.97
17	18	3.73, 3.40	7.13	3.56
19	20	3.70, 2.82	6.52	3.26
21	22	3.48, 3.08	6.56	3.28
23	24	3.00, 4.66	7.66	3.83
25	26	2.58, 2.94	5.52	2.76
27	28	2.85, 2.89	5.74	2.87
29	30	2.74, 3.34	6.08	3.04
31	32	3.39, 3.03	6.42	3.21

Mean square (between samples): 0.39
Mean square (within samples): 0.26
Mean square (replicates): 3.51 E-2
Error: 0.28

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.30	1.01 E-2	6.81	0
2	0.36	1.45 E-2	6.81	0
3	-1.14	0.15	3.37	3.44
4	-1.58	0.28	3.35	3.45
5	10.3	**12.1	3.72	3.08
6	5.62	3.59	3.58	3.23
7	2.08	0.49	3.47	3.34
8	0.96	0.10	3.43	3.37
9	0.96	0.10	3.43	3.37
10	-1.20	0.16	3.36	3.44
11	2.04	0.47	3.47	3.34
12	2.08	0.49	3.47	3.34
13	4.18	1.98	3.53	3.27
14	-1.58	0.28	3.35	3.45
15	-2.54	0.73	3.32	3.48

** - significant at 99% level
* - significant at 95% level

Table M. Sample data and analysis of variance of fall sample, Station 1.

SAMPLES	SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1 2	4.36, 4.08	8.44	4.22
3 4	4.46, 4.17	8.63	4.31
5 6	4.90, 4.97	9.87	4.93
7 8	5.21, 4.41	9.62	4.81
9 10	4.30, 3.91	8.21	4.10
11 12	3.97, 4.30	8.27	4.13
13 14	4.67, 3.63	8.30	4.15
15 16	3.89, 3.63	7.52	3.76
17 18	4.03, 4.22	8.25	4.12
19 20	4.44, 3.50	7.94	3.97
21 22	4.18, 3.86	8.04	4.02
23 24	4.57, 3.26	7.83	3.91
25 26	3.94, 3.59	7.53	3.76
27 28	3.79, 3.19	6.98	3.49
29 30	3.50, 3.55	7.05	3.52
31 32	2.13, 3.22	5.46	2.73

Mean square (between samples): 0.53
Mean square (within samples): 0.22
Mean square (replicates): 0.70
Error: 0.18

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.35	2.07 E-2	7.99	0
2	0.42	3.05 E-2	7.99	0
3	3.44	2.02	4.10	3.89
4	0.56	5.37 E-2	4.02	3.98
5	9.30	**14.8	4.29	3.71
6	9.78	**16.4	4.30	3.69
7	-2.22	0.84	3.93	4.07
8	-2.28	0.89	3.93	4.07
9	-1.88	0.60	3.94	4.06
10	-4.76	3.88	3.85	4.15
11	-4.08	2.85	3.87	4.12
12	-0.78	0.10	3.97	4.02
13	1.54	0.40	4.05	3.95
14	-0.34	1.98 E-2	3.99	4.01
15	-1.4	0.34	3.95	4.04

** - significant at 99% level
* - significant at 95% level

Table N. Sample data and analysis of variance of fall sample, Station 2.

SAMPLES	SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1 2	4.41, 3.42	7.83	3.91
3 4	3.83, 3.78	7.61	3.80
5 6	3.51, 3.90	7.41	3.70
7 8	3.74, 4.06	7.80	3.90
9 10	3.38, 3.23	6.61	3.30
11 12	3.28, 3.16	6.44	3.22
13 14	3.62, 3.46	7.08	3.54
15 16	3.36, 3.52	6.88	3.44
17 18	2.82, 3.13	5.95	2.97
19 20	3.27, 3.38	6.65	3.32
21 22	3.22, 3.47	6.69	3.34
23 24	3.04, 3.35	6.39	3.19
25 26	2.88, 3.25	6.13	3.06
27 28	2.57, 4.12	6.69	3.34
29 30	2.94, 3.80	6.74	3.37
31 32	3.52, 2.79	6.31	3.15

Mean square (between samples): 0.17
Mean square (within samples): 0.17
Mean square (replicates): 0.18
Error: 0.17

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.30	1.66 E-2	6.83	0
2	0.36	2.38 E-2	6.83	0
3	-0.33	2.03 E-2	3.40	3.42
4	-1.39	0.36	3.37	3.46
5	3.45	2.22	3.52	3.31
6	6.11	*6.95	3.60	3.22
7	-1.41	0.37	3.37	3.46
8	-0.81	0.12	3.39	3.44
9	0.73	9.92 E-2	3.44	3.39
10	0.89	0.15	3.44	3.38
11	0.03	1.68 E-4	3.41	3.41
12	3.83	2.73	3.53	3.29
13	0.63	7.39 E-2	3.43	3.39
14	2.57	1.23	3.49	3.33
15	1.39	0.36	3.46	3.37

** - significant at 99% level
* - significant at 95% level

Table O. Sample data and analysis of variance of fall sample, Station 3.

SAMPLES		SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1	2	4.76, 5.14	9.90	4.95
3	4	4.18, 4.53	8.71	4.35
5	6	3.65, 4.11	7.76	3.88
7	8	3.46, 4.84	8.30	4.15
9	10	3.79, 4.05	7.84	3.92
11	12	4.06, 4.18	8.24	4.12
13	14	4.44, 3.75	8.19	4.09
15	16	3.75, 5.45	9.20	4.60
17	18	3.94, 3.59	7.53	3.76
19	20	4.28, 3.81	8.09	4.04
21	22	4.29, 5.03	9.32	4.66
23	24	4.18, 8.32	12.5	6.25
25	26	3.95, 4.78	8.73	4.36
27	28	2.29, 6.00	8.29	4.14
29	30	3.93, 6.17	10.1	5.05
31	32	4.00, 3.38	7.38	3.69

Mean square (between samples): 0.81
Mean square (within samples): 1.37
Mean square (replicates): 6.28
Error: 1.04

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.38	4.46 E-3	8.76	0
2	0.46	6.30 E-3	8.76	0
3	-1.34	5.40 E-2	4.34	4.42
4	-5.42	0.88	4.21	4.55
5	4.14	0.52	4.51	4.25
6	-3.80	0.43	4.26	4.50
7	2.68	0.22	4.46	4.29
8	-4.84	0.71	4.23	4.53
9	-0.18	9.75 E-4	4.37	4.38
10	-1.88	0.11	4.32	4.44
11	7.90	1.88	4.62	4.13
12	-1.74	9.11 E-2	4.32	4.43
13	6.02	1.09	4.56	4.19
14	2.00	0.12	4.44	4.32
15	9.60	2.77	4.68	4.08

** - significant at 99% level
* - significant at 95% level

Table P. Sample data and analysis of variance of fall sample, Station 4.

SAMPLES		SAMPLE WEIGHT (mg/15 ml)	TOTAL	MEAN
1	2	4.66, 4.59	9.25	4.62
3	4	5.44, 4.93	10.4	5.18
5	6	4.28, 5.20	9.48	4.74
7	8	4.54, 5.84	10.4	5.19
9	10	4.29, 4.07	8.36	4.18
11	12	3.73, 4.23	7.96	3.98
13	14	4.51, 4.30	8.81	4.40
15	16	4.26, 4.28	8.54	4.27
17	18	4.72, 4.16	8.88	4.44
19	20	5.40, 4.71	10.1	5.05
21	22	4.63, 4.96	9.59	4.79
23	24	4.44, 4.47	8.91	4.45
25	26	3.47, 3.48	6.95	3.47
27	28	2.93, 4.35	7.28	3.64
29	30	3.01, 3.34	6.35	3.17
31	32	3.89, 3.20	7.09	3.54

Mean square (between samples): 0.77
Mean square (within samples): 0.21
Mean square (replicates): 0.11
Error: 0.21

FACTOR	COMPARISON	F-RATIO	MEAN 1	MEAN 2
1	0.38	2.06 E-2	8.64	0
2	0.46	3.03 E-2	8.64	0
3	-2.97	1.29	4.23	4.42
4	0.01	1.46 E-5	4.32	4.32
5	15.6	**35.7	4.81	3.83
6	7.99	**9.33	4.57	4.07
7	-1.59	0.37	4.27	4.37
8	-2.17	0.69	4.25	4.39
9	0.27	1.06 E-2	4.33	4.31
10	0.49	3.51 E-2	4.34	4.31
11	-2.55	0.95	4.24	4.41
12	-4.01	2.35	4.20	4.45
13	-2.67	1.04	4.24	4.41
14	1.41	0.29	4.37	4.28
15	1.09	0.17	4.36	4.29

** - significant at 99% level
* - significant at 95% level